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⑬ **Assay and sensing means for determining analyte.**

⑰ An assay employing a tracer comprising a ligand having a mercury label wherein mercury label is released from at least one of a bound or free tracer phase and interacts with a metal. Analyte is determined by a change in at least one property of the metal caused by such interaction. The invention also relates to a device for such an assay wherein mercury ions released from a free or bound tracer are caused to eventually amalgamate with a metal, and the presence and/or amount of analyte is determined by changes in the metal resulting from the eventual amalgamation which may be measured electrically or by other means.

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ASSAY AND SENSING MEANS FOR DETERMINING ANALYTE

Field of Invention

This invention relates to assays for determining an analyte within a whole blood or other liquid sample, and to sensing means used in conjunction with such assays. More particularly, this invention relates to assays for analytes which employ as a tracer in the assays a ligand labeled with mercury or labeled with mercury releasing means.

Prior Art

Various types of assays for analytes such as glucose, theophylline, phenytoin, cyclosporin, digoxin, cholesterol, and drugs of abuse (e.g. marijuana, cocaine) are known in the art. These assays may employ enzyme, radioactive, fluorescent, chemiluminescent, or other markers. The samples tested for analyte in conjunction with these assays include blood, cerebral spinal fluid, and urine. The types of assays described above, however, cannot be easily undertaken using a portable device which also enables one to obtain almost immediate results at the patient site and without centrifuging.

Until now, almost universal dependency on central laboratory testing has for years been and remains the standard in the industry.

Aside from central laboratory testing, several different systems or instruments are currently being employed in or have been proposed for the measurement of the many analytes found in blood. Each of these have shortcomings which restrict their breadth of use to areas other than whole blood assays. Almost all are not adaptable to small (pocket portable), simple to operate, or inexpensive one step instruments.

Even with the advent of some bedside and home testing (e.g. glucose), the major volume of tests are still performed in the clinical laboratory, primarily because of economic and skilled personnel restrictions.

BRIEF SUMMARY AND OBJECTS OF THE INVENTION

In accordance with one aspect of the present invention, there is provided an assay for an analyte which employs as a tracer in the assay a ligand labeled with mercury or mercury releasing means. The ligand used in producing the tracer is one which is bound directly or indirectly to the analyte or to a binder for the analyte. More particularly, an analyte is determined by an immunoassay or competitive or displacement binding procedure (sometimes referred to as a protein binding assay) wherein the tracer employed in the assay is labeled with mercury or mercury releasing means and the presence and/or amount of analyte is determined by determining a change in at least one property or characteristic of a metal which interacts with mercury directly or indirectly from the tracer.

The term mercury as used herein generally refers to mercury as a salt, and/or in an ionic form, and/or in a chelated state, etc. Thus, the mercury, as a label in the tracer or releasable by the tracer, may be present as a mercuric salt or mercuric ion and the term "mercury" as used herein encompasses such a mercuric salt and/or ion.

In an immunoassay procedure, there is formed a bound tracer phase in which the tracer is either bound to a binder for the analyte or directly or indirectly to the analyte and a free or unbound tracer phase. In accordance with an aspect of the present invention, the presence and/or amount of analyte is determined by determining a change in at least one property or characteristic of a metal which interacts with mercury directly or indirectly from the free and/or bound tracer phase.

Thus, the presence and/or amount of analyte may be determined by causing the mercury to interact with a metallic surface and then determining a change in one of the properties which result from such interaction.

The term interact, as used herein, encompasses amalgamation of the metal, as well as other reactions, reductions and/or oxidations. For example, the metal with which the mercury interacts may be part of an electrode or electrodes. Interaction of the mercury with the electrode or electrodes changes a property or properties of the electrode or electrodes such as adherence of protective surface film or layers. Changes in these protective films are manifest as current changes, changes in resistance or capacitance, electrolytic changes, etc., which may be measured. The presence and/or amount of analyte is determined by the

measurement of one or more changes in the properties of the metallic surface with which the mercury interacts. Other means of determining interaction of mercury with a metal include measuring the amount of heat generated by the exothermic interaction between the unprotected metal and its environment; measuring the change of mass of the metal; and visual and/or optical changes associated with the mercury dependent reactions between the metallic surface and its environment.

It is known that most metals, with the exceptions of iron and platinum, interact with mercury. In a preferred embodiment, however, the metallic surface which interacts with the mercury is aluminum.

The mercury may be released from the tracer by the use of an appropriate releasing agent, such as a nickel salt, e.g. nickel chloride, nickel sulfate, nickel nitrate. Other releasing agents may include, for example, salts of sodium, iron, lithium, manganese or magnesium. A preferred salt is nickel chloride. It is believed that the metal ion of the releasing agent displaces mercury from the tracer. It is also possible to release mercury from the tracer by a releasing agent which changes pH; for example, reduction of pH to below 1 releases mercury from a PEI carrier agent, hereinafter described.

In a preferred embodiment, the releasing agent, in particular NiCl_2 , is employed in conjunction with ammonium chloride which enhances the sensor signal.

In accordance with a second embodiment, the label present in the tracer is a mercury releasing agent that causes, directly or indirectly, the release of mercury in a subsequent step. For example, the tracer may be composed of a ligand covalently attached to a suitable enzyme. The enzyme selected interacts with a mercury containing substrate, thereby releasing mercury or mercury containing polymers. These compounds further interact either directly with a suitable metallic surface, or with further releasing agents as previously mentioned (nickel chloride and ammonium chloride) and then with the metallic surface.

Other objects and features of the present invention will be apparent from the detailed description taken with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a top plan view of a chain of frangible disposable sensors adapted to be inserted into an instrument;

Figure 2 is a cross-sectional view taken along lines 2-2 of Figure 1;

Figure 3 is a cross-sectional view taken along lines 3-3 of Figure 1;

Figure 4 is a fragmentary cross-sectional view taken along lines 4-4 of Figure 1;

Figure 5 is a cross-section taken along lines 5-5 of Figure 1;

Figure 6 is a fragmentary exploded perspective representation of components forming part of the sensor chain of Figure 1;

Figure 7 is a fragmentary plan elevational view, with certain parts removed for clarity, of the detent and release mechanism by which the sensor chain of Figure 1 is held in a desired position and by which the sensor chain of Figure 1 is advanced for removal of a used sensor and for disposition of an unused sensor for use;

Figure 8 is a fragmentary side elevational view schematically illustrating one way in which the sensor strip of Figure 1 is placed in selected electrical contact with an instrument's electrical connector;

Figure 9 is an enlarged top plan view of a lancet in spring form, shown in its loaded position, one of which is present in each of the sensors of the sensor chain of Figure 1;

Figure 10 is a side view of a further embodiment of an assay package, or sensing device, in accordance with the present invention;

Figure 11 is a cross-sectional view of the embodiment of Figure 10;

Figure 12 is a cross-sectional view of another embodiment of a sensor in accordance with the present invention, showing a space between a tracer pad and a releasing reagent pad which releases mercury;

Figure 13 is a cross-sectional view of still another embodiment of a sensor in accordance with the present invention depicting a water-soluble membrane between the tracer pad and the releasing reagent pad;

Figure 14 is a schematic view depicting the operation of the sensor shown in Figure 13;

Figure 15 is a cross-sectional view of an additional embodiment of a sensor in accordance with the present invention, showing a pad which contains a known amount of mercury ion;

Figure 16 is a cross-sectional view of one more embodiment of a sensor in accordance with the present invention, showing a dual flow configuration;

Figure 17 is a cross-sectional view of a further sensor embodiment;

Figures 18 and 19 are graphic representations of empirically derived glucose curves showing the sensor output current signal in respect to time; and

Figure 20 is a graphic representation of an empirically derived relationship between the sensor output current signal and the released tracer concentration.

DETAILED DESCRIPTION

General

Generally, the present invention relates to apparatus, and related methods, of quantitative detection of specific analytes in whole blood and other liquids at the sample acquisition site. Mercury (Hg) applied to Aluminum (Al) is known to destabilize the protective Aluminum Oxide (Al_2O_3) layer. The present invention involves a mercury-aluminum dependent sensor wherein resulting activity at the aluminum surface, due to the interaction of aluminum and mercury is proportional to the quantity test analyte in the specimen. Mercury is not found in analytical quantities in physiological fluids and has been found to possess significant advantages as an analyte tracer.

Competitive chemistry, employing, preferably in dry format, the analyte mercury tracer and a complementary set of reagents for each specific assay, is used to deliver measurable and analytically meaningful quantities of mercury to an aluminum surface to produce the sensor activity correlative to the quantity of test analyte.

All chemistry reagents and sensor components are preferably prepackaged within a disposable sensor housing. Low cost materials are used, producing a unique interplay between organic and inorganic chemistry. The tracer, as stated, is a mercury containing specific molecule or mercury-containing synthetic polymer, which is selectively released by a chemical reaction with the test analyte within the sample liquid and is transported a short distance within or by the sample liquid toward the aluminum surface. The released mercury then contact the aluminum surface. The released mercury interact with the aluminum surface and generate the activity, hereinafter more fully described, which is measured and a known function of the quantity or test analyte present in the specimen.

The presently preferred disposable sensor housing also comprises a lancet associated with each sensor used to pierce the skin allowing transcutaneous blood flow for droplet sample acquisition. The inclusion of a lancet as part of the sensor housing is desirable but not critical, since independent finger-piercing means can be used. The sensor receives the blood sample, processes the test, and emits electrical signals to the instrument.

The Sensor and Sensor Chain of Figures 1-9 and Instrument and Sensor Use

Reference is now made to Figure 1-9, which illustrate a presently preferred chain, packet or linkage of breakaway sensors, generally designated 350. The chain of sensors 350 comprises, as illustrated, eight sensors, each generally designated 352, each for quantitatively detecting a specific analyte in a liquid sample. The sensors of packet 350 are frangibly linked together, as later explained. The illustrated and described chain of sensors 350 and each sensor 352 thereof are specifically constructed for percutaneous, whole blood, long shelf-life applications. Each sensor 352, as explained in greater detail herein, is easily calibrated, usually at the factory. Operation of an instrument in conjunction with any one of the sensors 352 requires only two user steps, i.e. lancing of the skin and touching of the distal tip of the sensor to the resulting droplet sample of blood surfacing on the tip of the finger so lanced.

The chain of sensors 350 and each sensor 352 in particular comprise a top housing, generally designated 356, and a bottom main electrode-carrying plate, generally designated 358. The main electrode 360 (Figure 6) is adhered to and carried at the top surface 414 of the plate 358. Electrode 360 extends from near the distal to the proximal end 278 of the chain of sensors 350. This extension includes traversing about half way along the insertion tab 354. As also best shown in Figure 6, a pad-receiving plate, generally designated 362 and comprising spaced rectangular apertures 364, is contiguously disposed above the main negative electrode 360. Certain tracer and reagent pads 366 and 368, respectively, are sized and shaped to snugly fit in contiguous superimposed relationship within each rectangular aperture 364.

The chain of sensors 350 further internally comprises a sample flow-accommodating plate, generally

5 Adhered to the underside 374 are liquid sensing conductors or electrodes 376 and 378, which run the length of the linked sensors 352. The electrode 376 terminates at the interface between the first-to-be-used sensor 352 and distal insertion tab 354, while electrode 378 extends about midway through tab 354, for a purpose yet to be explained.

The top housing 356 of the chain of sensors 350 is generally of an inverted U-shaped configuration and surrounds the top and sides of the plates 358, 362 and 370. The bottom plate 358, at the edges thereof is contiguous with and integrally connected, by adhesive or other suitable techniques, to the side flanges 371 and 373 of the top housing 356. Thus, there is provided between the bottom plate 358 and the top U-shaped housing an elongated chamber 380, into which the plates 362 and 370 with electrodes 360, 376 and 378 are positioned along the entire length of the packet of sensors 350. Spaced lateral receiving compartments 382 (one for each sensor 352) exist in longitudinal spaced orientation. Each compartment 382 houses a spring-shaped lens, generally designated 384. See Figures 3, 4 and 9.

Top housing 356 is of injection molded synthetic resinous material, which is readily manipulable. 25
Housing 356 has spaced transverse V-shaped notches 390 interrupting the top surface 444 at the connection frangible sites 392 between the linked sensors 352. A similar notch 390 is interposed in the top surface of the housing 356 between the insertion tab 354 and the number one sensor 352. The bottom surface 393 of the lower plate 358 is likewise interrupted by V-shaped notches 394 which are respectively 30
located in vertical alignment with the notches 390 below each weakened frangible region 392. Each notch 394 extends across the bottom edges of flanges 371 and 373. Thus, when the user applies an upward or downward force on the insertion tab 354 or on an exposed user sensor 352, the housing 356, the plate 358 and the components therebetween will fracture at the frangible site 392.

The packet 350 of sensors 352, as shown in Figure 1, is entirely internally hermetically sealed at the time of manufacture and, preferably subject to conventional sterilizing techniques prior to distribution to the user. When a given sensor 352 becomes located at the opening of an instrument, the distal entry port 400 of the channel 388 (Figure 5) adjacent broken edges 402 and 404 becomes exposed for the first time. More specifically, when the preceding sensor 352, following use, is indexed forward and fractured from the packet 350 along its distal fragile site 392 to create exposed distal edge 402 of housing 356 and edge 404 of plate 358, the entry port 400 to capillary 388 is initially exposed. Thereafter, when the leading end of the instrument is placed so that a droplet specimen of blood on the fingertip of a medical patient is contiguous with the distal end 402/404 of an activated sensor 352 in alignment with capillary opening 400, blood will aspirate through the opening 400, along the capillary 388, through the associated aperture 372, into contact with electrodes 376 and 378, through the tracer and reagent pads 366 and 368 and into contact with the main negative electrode 360. See Figures 3 and 5. Testing for a specific analyte thereafter automatically occurs as the blood first infiltrates the pads 366 and 368 and thereafter makes contact with the surface of the main electrode 360, as explained herein later in greater detail.

The plate 370 partly covers the top of the pads 366 and 368 located in rectangular aperture 364 of plate 362 directly below each circular aperture 372 as well as the top surface 412 of plate 362.

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strip electrodes 360, 376 and 378 are treated for reactivity, as described later in this specification.

Reagent pad-receiving plate 362 is of hydrophobic synthetic resinous material and defines the previously described rectangular apertures 364. Plate 362 is bonded or otherwise suitably secured at the top surface 412 thereof and to the bottom surface 374 of the plate 370. The edges of the side plate 362 are bonded to the housing 356.

Bottom plate 358 is comprised of hydrophobic synthetic resinous material and is bonded or otherwise suitably secured along the surface 414 to the surface 416 of the plate 362. The plate 358 is further bonded or otherwise suitably secured along its side edges to the housing 356 in fluid tight relation. The described bonding of plates 370, 362 and 358 produces an integral and fluid sealed structure, as is illustrated best in Figures 3 and 5.

The main frangible electrode 360 is either bonded to or vacuum deposited on the top surface 414 of the plate 358. Electrode 360 extends from midway within the insertion tab 354 through each of the successive sensors 352. The synthetic resinous material from which bottom plate 358 is formed is readily manually frangible, to accomplish the objectives described above. The bottom plate has disposed in surface 414 a pair of grooves extending between each rectangular aperture 364 in plate 362 and an adjacent larger rectangular blind recess 420. The grooves 418 accommodate evacuation of air or other gas from within the associated capillary passageway 386, and the apertures 372 and 364 to accommodate capillary specimen flow, as described above. Rectangular blind recess 420 disposed in the top surface 414 of the plate 358, when disposed at the open distal end of an instrument is in communication with the atmosphere, i.e. through lancet opening 422 in the housing 356. See Figure 6.

As stated earlier, a spring-shaped lancet 384 is confined to the hollow lancet compartment 382 of each sensor 352 of the series packet 350 of sensors 352. The lower portion of each compartment 382 comprises one of the rectangular blind recesses 420 in top surface 414 of the plate 358. The top of each compartment 382 comprises an irregular recess surface 438. The front surface 432 of each compartment 382 is in vertical alignment with the recess 420. The trailing surface 434 is vertically out-of-alignment with the recess 420 forming a shelf or shoulder 436. A portion (the trigger 468) of the spring-shaped lancet 384 rests upon ledge 436. Most of the top surface of 438 of each compartment 382 is disposed within a horizontal plane, with the exception that it is interrupted by a downwardly projecting tab 440, the purpose of which will be later explained. Note that the tab 440 of each lancet compartment 382 is vertically aligned with a recess 442 disposed in the top surface 444 of the housing 356 and with lancet rod 454.

Reference is now made primarily to Figure 9 for the purpose of describing the spring-shaped lancet 384 which is contained in each compartment 382. The lancet 384, in its illustrated configuration, is formed of a single piece of high strength wire. The lancet comprises a proximal end 450 terminating in a blunt transverse proximal edge 452. Proximal end 450 comprises part of a linear segment or rod 454, which extends along the longitudinal axis substantially the full length of the lancet 384. Linear segment 454 terminates at 90 degree elbow 456, which merges with a reverse direction compression coil 458. The proximal end 460 of the coil merges with a diagonal segment 462 at elbow 464, which has an interior angle of about 45 degrees in respect the horizontal. Diagonal segment 462 merges at elbow 466 with transverse linear trigger portion 468. Trigger portion 468 in turn merges at ninety degree elbow 470 with a distal linear segment 472. Linear segment 472 is parallel to but off-set from linear segment 454. Nevertheless, linear segment 472 is disposed within the interior of the coil 458. Linear segment 472 terminates in a sharp beveled point 474.

Each lancet 384 is placed in its compartment 382 in a cocked position. More specifically, the spring configuration of each lancet 384, when placed in an unstressed state so that distal edge 452 is not contiguous and aligned with the trigger segment 468, due to the memory of the material from which the lancet 384 is made, will cause the trigger 468 to be forward of the proximal edge 452. At the time of manufacture of each lancet 384, the trigger segment 468 is forcefully retracted in respect to the rod 454 until the trigger 468 passes the proximal edge 452. The assembler then merely brings trigger 468 into contiguous alignment with proximal edge surface 452, much like cocking the string of an automated bow. See Figure 9. Note, in respect to Figure 4 that the trigger portion 468 rests upon the housing shelf 436 and the linear rod portion 454 is adjacent the tab 440 so that the cocked relationship between the trigger 468 and the edge 452 is not inadvertently changed. Thus, the trigger 468 and the lancet tip 474 are restrained from movement by the engagement between the edge 452 and the trigger 468. This retraction of the trigger 468 and the tip 474 is counter to the memory of the material from which the lancet 384 is made. When released, as hereinafter explained in greater detail, the energy contained within the cocked trigger 468 causes the tip 474 to be driven through the hole 422 in housing 356 to penetrate the finger of the medical patient to a predetermined distance adequate to provide a droplet blood sample at the fingertip shortly after lancing. Restated, the spring segment 458 of the lancet 384 is wound in such a way as to permit the inertia

of released caused lancet point 474 to overtravel its normal unstressed position to accomplish the aforesaid lancing. After lancing, the memory of the material from which the lancet is formed retracts the lancet tip back into compartment 382.

Thus, when the finger of the patient to be lanced is placed contiguously at the distal edge 402/404 of the exposed sensor in alignment with an instrument actuator, the user merely depresses the actuator to fire the cocked lancet 384 of the distal sensor 352. Once the energy within the lancet 384 has been dissipated, the memory of the material forming lancet 384 returns the tip 474 to an at-rest position within the compartment 382. In this way a used sensor with a used lancet concealed therein may be safely discarded.

It is to be appreciated that the interior of each sensor is hermetically sealed until the previously used sensor 352 is snapped off. At that point in time the capillary 386 and the lancet opening 422 are exposed for the first time.

The breakaway insertion tab 354 is primarily formed from frangible plastic. When the sensor packet 352 is initially and correctly inserted into an instrument the electronics of the instrument measure the resulting current flow through conductor 502. The value of this current flow is factory set and is related to lot-to-lot variations found in sensors fabricated in accordance with the present invention and is used to adjust the internal calibration of the instrument. This value remains in the instrument memory until updated by the current flow factor of the next sensor packet 350. When the breakaway insertion tab 354 of a packet 350 is snapped off prior to utilization of the first sensor 352, current flow between the electrodes 378 and 360 across conductor 502 stops.

With reference to schematic Figure 8, a small conductive strip 510 is bonded to the proximal end of the housing 356 at the base surface of the slots 276. Conductor 510 is disposed opposite to a pair of L-shaped, spaced contacts 512 and 514 carried by the plate 264. Conductivity between contacts 512 and 514 through conductor 510 is achieved when the sensor packet is fully inserted into the chamber of an instrument. At this time, when electrodes 512 and 514 are in contact with conductor 510, the sensor packet 350 has been correctly inserted into an instrument.

As can be seen in Figure 7, the indicated indexing may be controlled by the detent plate 310 and its detent 312. More specifically, each sensor packet 350 comprises one side edge 529 having spaced vertical notches 530 disposed therein in alignment with the notches 390 and 394 heretofore described. The at-rest position of detent plate 310 is such that, in the absence of a sensor packet 350 in tee chamber 244, detent 312 extends somewhat into the travel path used by the packet 350 when inserted and removed. Thus, when a packet 350 is inserted, the detent plate 310 is deflected transversely away from the displacement path of the packet. The memory of the material from which the spring plate 310 is made urges the detent in the direction of the notched edge surface 529 of the packet 350.

A resistor at 502 (Figure 2) is preferably placed between the liquid level sensor leads in the insertion tab 354 to indicate calibration values for the disposable packet 350. The value of that resistor is determined using the A/D converter at the time of first insertion of the disposable packet 350.

Quantitative Analyte Detection in Sensors

The foregoing description of the preferred embodiments of the sensor relates to a chain or packet of sensors of a definitive configuration. The present invention, however, embraces insertion and use of a single disposable sensor into the test instrument and variations in configurations. The ensuing description, directed to Figures 10-16, illustrates single sensors having configurations which vary in configuration but not in principle from the sensor embodiment of Figures 1-9.

In all illustrated configurations, each sensor functions to ascertain the quantity of a predetermined analyte in a whole blood or other liquid sample, accurately and automatically using a hand-held instrument and a droplet-sized sample.

Broadly, a mercury tracer site is infiltrated by a test analyte of unknown presence and quantity within the liquid sample. Competitively the tracer is released as a function of the quantity of the test analyte. The released mercury tracer is displaced by or within the liquid of the specimen to a site where reagent is available by which mercury, in ionic or salt form, from the released tracer is freed. The released mercury is transported by or within the liquid of the specimen to the surface of an aluminum body, where the released mercury interacts with said surface, measurably changing the characteristics thereof.

Reference is now made to Figures 10 and 11, which illustrate a sensor 552, in accordance with the present invention. Sensor 552 is comprised of a housing 570. Electrical connections 602 are illustrated as protruding from the one end 600 of housing 570. See Figure 11. Extending from a second opposed end 604 of housing 570 is capillary tube 614 having an inlet opening 616.

A sample enters inlet opening 616 of capillary tube 614. The sample travels upwardly through tube 614 by capillary movement. When the sample reaches and bridges the ends 623 and 625 of electrodes 621 and 622, which are connected to the electrical connections 602, which may be sensed to indicate that a sufficient volume of sample has entered the sensor. Thus, the capillary tube size and electrode spacing is selected to require sufficient liquid volume in the specimen to trigger the initial signal and permit an accurate analyte test. The sample, therefore, acts as a conducting medium between electrodes 621 and 622, at the distal ends 623 and 625, which ends are exposed at the proximal end 617 of capillary tube 616. Electrode 621 also serves as a positively charged electrode of the sensor. Electrode 622 serves only as the negative electrode during the described liquid sensing phase. The sample then may contact a thin distributor pad 618 (optional) which spreads the flow of sample evenly across a tracer material pad 620.

The sample passes through distributor pad 618 and contacts a thin tracer pad 620. Tracer pad 620 is made of a material which permits or facilitates capillary flow of the sample through the tracer pad. Tracer pad 620 contains the tracer, which may be a graft copolymer of polyethyleneimine and polyethylene glycol having a mercury label, said copolymer also being attached to an appropriate ligand. The ligand used is dependent on the type of assay employed and the analyte being assayed. In a preferred embodiment, tracer pad 620 also contains a binder for analyte as well. In alternative embodiments, not shown, the tracer and binder may be on separate pads, the disposition of which being dependent upon the type of assay employed.

Contact of the sample with tracer pad 620 thus sets in motion the assay process. Analyte, if present, competes with the tracer to release an amount of tracer which is a function of the analyte concentration. The released tracer, containing the mercury label, is freed to move from the tracer pad 620 to the releasing reagent pad 624, which releases mercury ions from the tracer. As tracer is released from tracer pad 620, the tracer and sample move upwardly through the sensor and contact releasing reagent pad 624. The pad 624 is preferably impregnated with a nickel salt, most preferably nickel chloride and ammonium chloride. Releasing reagent pad 624 is also made of a material which will facilitate capillary flow of the sample. As the sample and tracer contact the reagent pad 624, the nickel chloride releases mercury ions from the tracer.

After the mercury is released from the tracer upon contact with reagent pad 624, the mercury ions contact a negatively charged electrode 626, which is also connected to electrical connectors 602. Electrode 626 is made of aluminum with a surface film of aluminum oxide/hydroxide plus surface-modifying agents. Upon contact with electrode 626 by mercury released from the tracer by releasing reagent pad 624, the mercury and aluminum surface interact eventually forming a mercury-aluminum product on the surface of the electrode 626.

In a preferred embodiment, the positive and negative electrodes are operated at about 0.2 volt. In general, the system, as presently configured, should not be operated at above 0.5 volt. In general, the positive electrode is smaller than and must be wet before the negative electrode. In a preferred embodiment, both electrodes are formed from aluminum.

The electrode 626, as above stated, has a barrier or protective surface layer, of aluminum oxide or Al_2O_3 . When water is present, as during electrode surface modification, an additional porous $Al(OH)_3$ layer forms on the Al/Al_2O_3 layer. The mercury, upon contact with the electrode 626, interacts or reacts with the surface of the aluminum electrode to destabilize the protective oxide/hydroxide layers and eventually form a mercury-aluminum amalgam. The changes produced by or leading to the formation of the amalgam are measured, preferably electrically. The changes which can be measured include electrolytic changes, resistance changes, and capacitance changes. The measurements of these changes in the electrode properties can be used to determine the amount of analyte present in the sample being assayed.

Also contained in the sensor is vent hole 628, positioned at the region where flow of sample occurs in the sensor between releasing reagent pad 624 and electrode 626, providing for the release of air and/or gas so that the flow of the sample will not be impeded.

The pads employed in the sensor are those capable of holding the particular reagent and which induce or permit capillary flow. The pads may be formed from a porous material and as representative examples there may be mentioned porous paper, wettable cloth (woven and non-woven), porous hydrophilic plastics, etc.

The reagents may be simply soaked or dispensed onto the pad then oven dried, as in the case of the releasing agent. In the case of binder and/or tracer, the reagent may be applied to the pad and lyophilized. In the case of a binder, in some cases it may be necessary to bond or covalently attach the binder to the pad, rather than simply soaking the pad with the binder.

Instead of using a pad for supporting the binder and/or tracer, such reagents could be included in a capillary tube or other support capable of inducing flow. For example, tracer may be lyophilized on the

interior wall of a capillary tube, and a sample introduced into the tube for subsequent flow and contact with a binder.

In one alternative embodiment, shown in Figure 12, the configuration, operation, and members are identical to those of the sensor of Figure 17, except as follows.

5 Disposed between tracer pad 620 and releasing reagent pad 624 is a space 631. Space 631 enables the device to be programmed so as to allow the sample and tracer to undergo an incubation period for a predetermined time. The predetermined incubation time allows for more control of the flow of the free tracer into the releasing reagent pad 624, and the subsequent generation of a more consistent signal from the device upon the release of mercury.

10 At the end of the predetermined incubation period, the device becomes compressed so as to enable tracer pad 620 and releasing reagent pad 624 to contact each other, thus enabling the flow of sample and tracer from tracer pad 620 to releasing reagent pad 624.

Another alternative embodiment, shown in Figures 13 and 14, is the same in operation, members and junction as those of Figure 18, except as follows: A water-soluble membrane 630 is disposed between 15 tracer pad 620 and reagent releasing pad 624.

Membrane 630 is of a thickness such that it dissolves after a predetermined time period. Once membrane 630 is dissolved, the sample and tracer then contact releasing reagent pad 624 and proceeds as described earlier. Membrane 630, therefore, provides for a limited time sample flow separation between 20 tracer pad 620 and releasing reagent pad 624, thereby providing a predetermined incubation time of the sample and tracer within the tracer pad 620, the advantages of which have been described above.

Figure 15 depicts an embodiment, which is known as a self-referencing sensor. A sample enters inlet opening 616 of capillary tube 614 and travels by capillary flow, as described above. The sample then 25 contacts bridging electrodes 621 and 622 at distal ends 623 and 625, conduction through which indicates a sufficient volume of sample has entered the sensor. Two sensors are provided by paths A and B with a single positive electrode 621.

As the sample moves by capillary flow through spreading pad 618, the flow of the sample is divided into two flow paths, A and B, by impervious divider 634. In flow path A, the sample flows from spreading pad 618 and contacts tracer only pad 632. Since tracer only pad contains no binder, the flowing sample 30 dissolves all the tracer present in the pad. The sample flows through tracer only pad 632 and then contacts releasing reagent pad 624, which releases mercury ions from freed tracer, as described above. The released mercury ions then contact aluminum electrode 626 and eventually amalgamate with the aluminum. Changes at the surface of the electrode 626 are then measured electrically through electrodes 602.

Along flow path B, the sample leaves spreading pad 618 and contacts tracer pad 633 which also 35 contains binder for the analyte, whereby tracer is released from the tracer pad 633 as described above. The sample plus tracer flows by capillary action into releasing reagent pad 624, which releases mercury.

The released mercury ions then contact aluminum electrode 626, whereby the mercury eventually amalgamates with the aluminum and changes of the surface of electrode 626 are measured electrically. The two flow paths, A and B, therefore, provide for an internal calibration or reference of the changes 40 generated by the eventual amalgamation of mercury with the aluminum surfaces of electrodes 626 and 626. This comparison may then be used to determine the presence and/or amount of analyte in the sample.

Also present are vent hole 628, along flow path A, and vent hole 628, along flow path B, which provide for release of internal air and/or gas from the sensor, as described above.

45 A self-calibrating device is shown in Figure 16. A sample enters the device through inlet 716 of capillary tube 714. The sample moves through tube 714 by capillary movement and contacts bridging electrodes 721 and 722 at 623 and 625, which are connected to electrical connectors 702. Electrical connection of the electrodes by the liquid sample indicates a sufficient volume of sample has entered the device.

50 After the sample has entered tube 714, the flow of the sample is divided into two flow paths, C and D, by impervious divider 732. In flow path C, the sample contacts spreading pad 718, which evenly distributes the sample for contact with blank pad 730. Blank pad 730 has no chemical function, but serves to balance the time flow of the flow paths. The sample in flow path C travels by capillary flow through blank pad 730 and then contacts tracer pad 720 which also includes binder for the analyte, which releases tracer into the sample, as hereinabove described. Sample and released tracer travel through tracer pad 720 by capillary 55 flow, and then contact releasing reagent pad 724, which releases mercury ions from the released tracer, as explained. The mercury ions then contact aluminum electrode 726, whereby the mercury eventually amalgamates with the aluminum surface and changes in the surface of the electrode are measured electrically, as hereinabove described.

Along flow path D, the sample contacts spreading pad 718', which spreads the sample evenly upon pad 731. Upon contact with pad 731 by the sample, pad 731 releases a known concentration of analyte to the sample. The sample and the known concentration of analyte flow through pad 731 and contact tracer pad 720 which also includes binder for the analyte, which upon contact with the sample and known concentration of analyte, releases free tracer into the sample, as hereinabove described.

A dual assay sensor is also represented by Figure 16. In this embodiment two separate assays may be performed simultaneously. Operation is similar to that described for the self-calibrating device except: each pathway contains reagents specific for the intended assay. It is appreciated this embodiment, while describing a dual assay sensor, may be expanded with incorporation of additional assay flow paths to encompass performance of multiple simultaneous assays.

In one alternative embodiment, shown in Figure 17, a sample enters inlet opening 640 of capillary tube 642 and travels upwardly through tube 642 by capillary movement. The sample then contacts and bridges electrodes 644 and 646 at exposed distal ends 648 and 650, respectively. Electrodes 644 and 646 are connected to the electrical connections 652. Electrical conduction occurs to indicate when a sufficient volume of sample has entered the sensor. Electrode 644 also serves as a positively charged electrode of the sensor. The sample then contacts spreading pad 654 of the sensor, which spreads the flow of sample evenly across tracer pad 656. The sample contacts and is absorbed into tracer pad 656 which also includes binder for the analyte, which starts the assay process, as described above, whereby analyte and tracer compete to release an amount of tracer which is a function of the analyte concentration.

Disposed between tracer pad 656 and releasing reagent pad 658 is a mercury-containing substrate pad 660. Sample and tracer flow into mercury containing substrate pad 660 by capillary action. The tracer, in this embodiment labeled with a mercury copolymer releasing agent, most preferably an enzyme, causes cleavage and/or release of mercury copolymer containing substrate subunits, which dissolve into the sample present in the mercury-containing substrate pad 660. The liquid contacts releasing reagent pad 658 resulting in liquid flow from mercury-containing substrate pad 660 to releasing reagent pad 658. As hereinabove described the enzyme label may be alpha-amylase, the substrate may be insoluble amylose (which is cleaved by alpha-amylase) to which is attached mercury containing copolymer of the type hereinabove described. Releasing reagent pad 658, which is impregnated with a salt such as nickel chloride and ammonium chloride, enables mercury to be released from the soluble substrate subunits. The mercury then contacts negatively charged aluminum electrode 662, whereby the mercury interacts with the aluminum surface and changes in the surface of the aluminum electrode are measured electrically. The device also contains a vent hole 664, which is positioned between releasing reagent pad 658 and electrode 662 so as to provide for the release of air and/or gas from the device.

The examples contained herein are by way of example only and it is understood combinations arising therefrom are within the scope of these teachings. For example, an enzyme tracer-substrate system may be used in conjunction with water soluble membranes or deformable spaces to provide for controlled incubations (sample-tracer and/or tracer-substrate) and for self-referencing and/or self-calibrating features.

Although it is preferred that analyte be determined by electrical means resulting from mercury-induced protective surface film destabilization on the electrode surfaces, it is also contemplated that the presence and/or amount of analyte may be determined by other means resulting from mercury-induced protective surface film destabilization. These include heat generated from the exothermic reaction between unprotected aluminum and its environment, measurement of the change of mass of unprotected aluminum and its environment, and optical or visual quantification of surface reflectivity or other surface changes resulting from reaction between unprotected aluminum and its environment.

The preferred embodiments are not limited as hereinabove described, and changes may be measured other than by electrical means.

Thus, for example, a thin layer of aluminum (for example, a few atoms thick) may be deposited on a transparent support (such as glass or plastic) and a change in reflection from the aluminum surface resulting from interaction with mercury released from bound and/or unbound tracer may be determined.

The change in reflectivity is a result of mercury interaction and destabilization of the protective films present in the aluminum surface. Without the protective surface films, the aluminum reacts rapidly with its environment, in this case the water present in the sample. The reflectivity of the aluminum therefore changes rapidly due to changes in aluminum layer thickness brought about by mercury induced surface reactions. It is appreciated that the aluminum surface reflectivity may be measured from the transparent support side, thereby eliminating light attenuation that may occur in the liquid sample.

As a further alternative an aluminized thermistor may be employed, which would be sensitive to interaction between the aluminum surface and the mercury released from the tracer.

While sensors embodying the present invention are accurately characterized as not enzyme dependent,

it should be appreciated from the foregoing that it is within the purview of the invention to use one or more enzymes to enhance the quantity of mercury ions reaching the negative aluminum electrode thereby increasing the sensitivity of the sensor.

5 Preferred Binder, Tracer, Assay Chemistry and Objectives Thereof

Assays which may be employed in accordance with the present invention include competitive assays, sandwich assays, and indirect sandwich assays.

10 In a competitive assay, the analyte and the tracer for the analyte compete for a limited number of binding sites on a binder, which is specific for the analyte and the tracer. The tracer, in accordance with the present invention, comprises the analyte or an appropriate analog thereof having a mercury or mercury releasing label.

In a sandwich assay, there is formed a complex of analyte bound to both a binder for the analyte and a tracer comprised of a ligand bound with mercury or mercury releasing means.

15 In an indirect sandwich assay, there is formed a complex of analyte bound to a first and second binder for the analyte and tracer bound to the second binder wherein the tracer is comprised of a ligand bound by the second binder labeled with mercury or mercury releasing means.

The sandwich or indirect sandwich assay may be accomplished by the forward, reverse or simultaneous technique.

20 In such assay procedures, there is formed a bound tracer phase and a free tracer phase and, in the assay, a change in at least one property of a metallic surface which interacts with the mercury is determined in the free and/or bound tracer to determine the presence and/or amount of analyte in a sample.

The binder used in the various assays is determined by the assay procedure. The binder is generally specific for at least the analyte and, in a competitive assay, the binder may be specific for both analyte and tracer. The binder may be an antibody and the antibody may be a monoclonal or polyclonal antibody. The binder may also be a naturally occurring substance. The binder may also be an antigen in the case, for example, where the analyte is an antibody. The selection of a suitable binder is deemed to be within the skill of the art from the teachings herein.

30 Similarly, the ligand portion of the tracer is determined by the assay procedure. Thus, for example, If the ligand of the tracer is to be bound to the analyte, the ligand may be an antibody (monoclonal and/or polyclonal) or in the case where the analyte is an antibody, the ligand portion of the tracer may be an antigen or an antibody. In an indirect sandwich assay, the ligand portion of the tracer may be an antibody or immunoglobulin bound by the second binder. The selection of a suitable ligand portion for the tracer is deemed to be within the scope of those skilled in the art from the teachings herein.

The binder for the analyte used in the assay may be supported on a solid support.

35 In some cases, the analyte may be captured on a solid support without the use of a binder for the analyte, and then the tracer is bound directly or indirectly to the analyte. Analyte is determined by measuring or determining a change in at least one property of a metallic surface which interacts with mercury derived from free and/or bound tracer.

40 In a preferred embodiment the ligand portion of the tracer is preferably labeled with mercury by the use of a carrier group comprised of a first portion to which mercury may be releasably attached and second portion to which the ligand is attached. In accordance with a preferred embodiment, the carrier group is one to which a plurality of mercury may be attached whereby each tracer molecule is labeled with a plurality of mercury. Thus, the carrier group has a backbone to which is releasably attached a plurality of mercury whereby a single tracer molecule includes a plurality of mercury.

45 As representative examples of groups which can releasably link mercury there may be mentioned primary, secondary and tertiary amines which may be present as a single group or multiple groups for releasably linking mercury. Acid groups may also releasably bind mercury. Sulfhydryl groups are also known to bind mercury; however, it is more difficult to release mercury ions from sulfhydryl groups. Although, in a preferred embodiment, the mercury is linked to groups from which it may be displaced by other metals, it is possible to link mercury to a tracer in a manner such that mercury is released therefrom by cleaving with an appropriate agent.

50 In accordance with one embodiment, there is provided a carrier group which is a copolymer of a polymer which provides multiple linking sites for mercury and a polymer which provides hydrophilic groups to render the copolymer, including attached mercury, water soluble. The hydrophilic polymer is preferably a non-ionic polymer to eliminate possible interference with mercury used as a label. As representative examples, there may be polyethylene glycol, or polymers which provide polyhydroxy groups, such as

sorbitol, sugars, etc. The polymer to which multiple mercury labels may be attached may be, for example, polyacrylic acid or a polyimine, with polyethyleneimine being preferred.

The copolymer is preferably a graft copolymer of the two polymers.

- 5 The copolymer which includes a hydrophilic polymer portion and a polymer portion which is capable of releasably linking or attaching mercury may be modified to include groups capable of reacting with an appropriate functional group of the ligand portion of the tracer, or substrate suitable for use with labeling enzyme.

- In accordance with a preferred embodiment of the invention, there is provided a carrier group for attaching a plurality of mercury to a ligand, which is a copolymer of polyethyleneimine polymer and a hydrophilic polymer; in particular a polyoxyalkylene with polyoxyethylene being preferred. The polyoxyalkylene portion of the copolymer is provided with an end group which is capable of being linked to the ligand portion of the tracer; for example an amino, carboxy, sulphydryl, or hydroxy group. In a preferred embodiment, the end group is a primary amine, derived from an epoxy group.

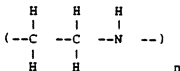
- 15 The polyethyleneimine portion of the copolymer serves as a backbone to which mercury may be attached and the hydrophilic polymer portion of the copolymer functions to provide compatibility with an assay system, and in particular blood as well as providing means for establishing specific assay identity by ligand attachment.

- Although the present invention is not limited to any theoretical reasoning, it is believed that the hydrophilic polymer portion forms an outer hydrophilic shell around the polyethyleneimine polymer to which the mercury is attached, thereby providing compatibility with water-based assay systems.

- 20 Although, in a preferred embodiment, the mercury label is attached to a ligand through a carrier group and in particular a copolymer of the type described, it is to be understood that the overall invention is not limited to such coupling.

- The tracer, in a preferred embodiment is comprised of a mercury label, a graft copolymer of polyethyleneimine and polyoxyethylene and a ligand attached to the polyoxyalkylene portion of the copolymer. A mercury label is displaceably bonded to the polyethyleneimine portion of the copolymer, and at least one epoxy group attached to the polyoxyalkylene portion may be opened so as to enable bonding to a ligand.

- 30 The polyethyleneimine portion of the copolymer has a structure as follows:

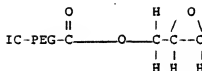


- 35 The polyethyleneimine polymer is capable of interacting with a mercury-containing compound, most preferably mercuric acetate, to link the mercury to the polyethyleneimine polymer. Although applicants do not intend to be limited to any theoretical reasoning, it is believed that the mercury is linked as mercuric ions in a chelated form.

- 40 In this example, a plurality of mercury may be reversibly linked to the polyethyleneimine polymer along the length of the polymer backbone. In a preferred embodiment, the polyethyleneimine polymer has a molecular weight of about 50,000. Compounds other than mercuric acetate may be used to attach the mercury to the polyethyleneimine polymer, but mercuric acetate is preferred because of its greater solubility.

- 45 It has been determined that the polyethyleneimine polymer with the mercury label, as described above, is not water soluble. Therefore, it is desirable to surround the polyethyleneimine with a hydrophilic surface.

- To accomplish the above, the polyethylene glycol polymer is grafted to the polyethyleneimine polymer. 50 The polyethylene glycol polymer preferably has a molecular weight of up to about 5,000, most preferably at about 1,000. The polyethylene glycol polymer, which is to be attached to the polyethyleneimine polymer, is prepared by coupling polyethylene glycol (PEG) with carbonyldiimidazole (CDI) to form PEG-di Carbonyl Imidazole (PEG-DCI), which is a structure with functional molecular end groups. To prevent cross-linking through the reactive imidazole rings on both ends of the PEG-DCI polymer, an epoxy compound, glycidol, 55 is reacted with PEG-DCI to form a compound as follows:



The epoxide group is introduced into the PEG-DCI to prevent cross linking since the Carbonyl Imidazole (CI) groups will react with -OH, -NH₂, etc. A group is reacted with one end of the molecule that is not reactive to CI groups; is not reactive with -NH- groups, yet can be made reactive after the graft copolymer is formed. Glycidol, being both an alcohol and epoxide, reacts with the PEG-DCI through the -OH. Although the epoxide will react with the -NH- of the PEI, it reacts considerably more slowly than do the CI groups. Cross linking is delayed due to the slow reactivity of the epoxide at room temperature with PEI.

It can be seen that the reaction of PEG-DCI with glycidol enables an epoxy group to be placed at the end of the polyethylene glycol polymer chain. The polyethylene glycol polymer chain is then attached to the polyethyleneimine polymer. High salt concentrations may be used to control polymer conformation in solution. The resulting copolymer may be visualized as a polyethyleneimine central core surrounded by a polyethylene glycol shell with epoxide groups on the outside of the shell. The polyethyleneimine/polyethylene glycol graft copolymer is further reacted with ammonium hydroxide in order to open up the epoxy groups to produce an active site, which, in this embodiment is an amino moiety, onto which may be bonded an appropriate ligand. The epoxide may also be opened with other reagents to result in different end groups such as -OH, -COOH, -SH, which may be further modified by known techniques for ligand attachment.

Rather than glycidol, other agents may be employed to prevent cross linking yet allow future attachment.

A maleimide may be introduced into the PEG either before or after reaction with CDI. A molecule of the general form

A - PEG - B

where A is an amine reactive site (CI, active ester, etc.) and B is a non amine reactive, -SH or other reactive site (maleimide, etc.). A reacts with PEI while B cannot. B is then later reacted with a ligand of choice. Note in this case the reactive site in the ligand is something other than an amine. In the preferred method, previously described, A is CI and B is an epoxide from glycidol. While B will react slowly with -NH-, B is converted to -NH₂ rapidly (prior to cross linking). The ligand, then, must be modified to contain a reactive group (CI, active ester, etc.) that preferentially reacts with the terminal -NH₂ groups (stronger nucleophile, better position).

In this preferred embodiment, the ligand should not itself contain amines, as cross linking may occur. For protein attachment, a maleimide reaction, as described, or by reaction of an NHS or CI maleimide may be used.

Thus, for example, glucose tracer may be produced wherein maltose is dissolved in dry DMF (aprotic solvent) and CDI reacts to form Maltose-Cl. This is a timed reaction, as the -Cl groups will further react with other -OH in Maltose and cross link.

PEI-PEG-NH₂ + Maltose-Cl →

PEI-PEG-NH- $\overset{\text{O}}{\underset{\text{O}}{\text{C}}}$ -O-Maltose.

Maltose is used to provide an unmodified glucose-like end after the coupling reaction. A mercury label may then be linked to the PEI.

In forming a theophylline tracer 3-(4-carboxybutyl)-1-methylxanthine (Clin. Chem. vol. 30, #7, 1984, page 1175) is converted to the active ester form by known techniques. This reacts directly with PEI-PEG-NH₂ to form the tracer. The resulting product may then be labelled with mercury.

A phenytoin tracer may be produced in which phenytoin active ester is formed by known techniques, and is reacted directly with PEI-PEG-NH₂.

A protein may be coupled to form a tracer in which the ligand is a protein. PEI-PEG-NH₂ is reacted with a commercially available bifunctional coupling agent (NHS-maleimide) to form PEI-PEG...maleimide.

The PEI-PEG-maleimide reacts directly with free sulphydryl groups in proteins. It is possible to introduce -SH groups into proteins that do not possess available -SH groups by known techniques.

The tracer may then, in accordance with a preferred embodiment, be placed on a pad which is contained within a housing or package as hereinafter described. Also placed on the pad may be a binder for an analyte.

In accordance with a further embodiment of the invention, there is provided a substrate for a mercury releasing agent, said agent being attached to a ligand and thereby forming a tracer. The substrate releases mercury or mercury-containing polymer in proportion to the amount of tracer present.

In a preferred embodiment of this system, the tracer label (mercury releasing agent) is an enzyme that causes cleavage or dissolution of insoluble polymeric materials. Examples of such enzymes include cellulases, dextranases, amylases, agarases, proteolytic enzymes, etc.

Functionally, the substrate is comprised of mercury containing copolymer covalently attached to an insoluble polymeric substrate. The enzyme is attached to the ligand through known techniques, and is selected to be reactive with a modified, mercury polymer containing substrate.

By way of example, bacterial alpha-amylase is readily available. It is known to cleave both soluble and insoluble fractions of starch at intermediate points in the polymeric starch structure. Amylose (insoluble fraction in starch) is thought to be a linear polysaccharide of several million molecular weight. Amylase is known to cleave the insoluble amylose molecule into smaller, soluble fractions by reaction at intermediate points along the linear polysaccharide. Dye molecules are readily attached to amylose without limiting amylase activity. Amylase activity may be measured by quantification of soluble dye content after reaction. In a similar manner, mercury-containing PEI-PEG copolymer may be covalently attached to insoluble amylose. Amylase activity cleaves the insoluble amylose molecule into smaller, soluble units, each unit containing mercury-containing PEI-PEG copolymer. The mercury is released from these soluble, copolymer-containing polysaccharide units with agents, such as nickel chloride/ammonium chloride, as previously discussed. This second embodiment provides chemical amplification in the system, since a single tracer molecule is able to cleave many mercury copolymer-containing polysaccharide units per minute.

The preparation of the mercury-containing insoluble substrate generally follows the principles outlined herein for preparation of copolymer tracers. By way of example, a substrate for alpha-amylase may be prepared by activating insoluble amylose according to generally known techniques, for example, with CDI in dry DMF. PEI is allowed to react with the activated amylose, after which the amylose-PEI is reacted with methoxy-PEG-Cl to improve liquid solubility, as previously discussed. Methoxy PEG-Cl is used rather than PEG-DCI so as to eliminate cross-linking.

Alternatively, Cl-PEG-NH₂, from Cl-PEG-glycidol, may be used. The ratio of mercury copolymer to amylose is adjusted to allow amylase activity while providing for high molecular amplification.

In accordance with another aspect of the present invention, a reagent kit or package for determining analyte is provided, which includes a tracer comprised of ligand labeled with mercury or mercury-releasing means and a binder for at least the analyte. The binder may be supported on a solid support. The reagent kit or package may also include other reagents, such as a substrate pad and/or a releasing agent for releasing mercury label. The kit may also include standards, buffers, etc.

In accordance with yet another aspect of the present invention, there is provided a device or product for determining analyte which includes a tracer comprised of a ligand labeled with mercury, a binder for at least the analyte, and metallic surface which interacts with mercury and has at least one property thereof changed by such interaction. Aluminum is particularly preferred. The metal, tracer and binder are incorporated into a container or housing in a manner such that sample may be added to the housing or container and the presence and/or amount of analyte determined.

In accordance with yet another aspect of the present invention, there is provided a device or product for determining analyte which includes a tracer comprised of a ligand labeled with a mercury releasing means, a binder for at least the analyte, a mercury containing substrate, and a metallic surface which interacts with mercury and has at least one property thereof changed by such interaction. Aluminum is particularly preferred. The metal, tracer, binder, and substrate are incorporated into a container or housing in a manner such that sample may be added to the housing or container and the presence and/or amount of analyte determined.

In accordance with a preferred embodiment, the metallic surface which is to interact with mercury is present in the form of an electrode and mercury is released from a bound and/or free tracer or cleaved substrate phase formed in the assay and caused to interact with the electrode to change at least one property thereof.

In accordance with a particular embodiment, there is provided in a container or housing an electrode which is formed from a metal the surface of which interacts with mercury, a binder for the analyte, a tracer comprised of a ligand labeled with mercury and a releasing agent for releasing mercury label from the tracer, all of which are situated and positioned in the housing such that upon adding of sample, there is formed a bound tracer phase and a free tracer phase, with mercury being released from one of the free or bound tracer phase (preferably the free tracer phase) and caused to come into contact and interact with the

electrode surface to change at least one property thereof.

In accordance with a preferred embodiment, there is provided a housing having an inlet means for receiving a sample suspected of containing analyte, a means for containing the mercury labeled tracer, means for containing a binder for at least the analyte, and means for containing a releasing reagent for releasing mercury label from the tracer and a metal electrode formed from a metal the surface of which interacts with mercury. The various means are situated in the housing in a manner such that analyte, binder, and tracer are contacted to form a bound and free tracer phase, with free tracer phase coming in contact with the releasing agent, and released mercury then coming in contact with the electrode.

In accordance with a further particular embodiment, there is provided in a container or housing an electrode which is formed from a metal the surface of which interacts with mercury; a binder for the analyte; a tracer comprised of a ligand labeled with mercury releasing means, a mercury containing substrate, a releasing agent for releasing mercury label all of which are situated and positioned in the housing such that upon addition of sample, there is formed a bound tracer phase and a free tracer phase, with mercury being released as a function of either the free or bound tracer phase (preferably the free tracer phase) and caused to come into contact and interact with the electrode surface to change at least one property thereof.

In accordance with a further preferred embodiment, there is provided a housing having an inlet means for receiving a sample suspected of containing analyte, a means for containing tracer, means for containing a binder for at least the analyte, means for containing a mercury containing substrate, and means for containing a releasing reagent for releasing mercury and a metal electrode formed from a metal the surface of which interacts with mercury. The various means are situated in the housing in a manner such that analyte, binder, and tracer are contacted to form a bound and free tracer phase, with free tracer phase coming in contact with a mercury containing substrate, the products of which contact a releasing agent, and released mercury comes in contact with the electrode.

The container or housing may also further include a means for determining a sufficient volume of sample within said inlet means. The inlet means may comprise a capillary tube.

The tracer-containing means may comprise solid absorbent such as a pad impregnated with at least said tracer, and the releasing reagent-containing means may comprise a solid absorbent such as a pad impregnated with the releasing reagent. The tracer-containing means may also include a binder for the analyte. The tracer-containing means and the releasing reagent containing means may each be comprised of an absorbent material that facilitates capillary flow through the material.

In a preferred embodiment, the metal surface which interacts with the mercury is aluminum.

The means for determining the presence of a sufficient volume of sample within said inlet means may include electrical means which are bridged by the sample being assayed.

The aluminum electrode which is to be contacted with the released mercury, normally has a protective layer of Al_2O_3 , which prevents rapid oxidation of the aluminum. Mercury serves to destabilize the Al_2O_3 layer. The changes in the electrical characteristics of the surface of the electrode, when the aluminum electrode is contacted by and the aluminum interacts with the mercury, may be measured in order to determine the presence and/or amount of analyte.

When water is present, an additional porous $Al(OH)_3$ layer will form on the Al/Al_2O_3 surface. mercury and/or its ions are relatively permeable in the $Al(OH)_3$ layer; thus, the interaction can proceed in aqueous solutions. It must be noted, however, that if the porous aluminum hydroxide layer is too thick, the sensitivity of the interaction of the mercury with aluminum will be markedly diminished. The aluminum electrode, therefore, is preferably subjected to treatment which will provide for a suitable oxide/hydroxide thickness to enable adequate sensitivity of the interaction of the aluminum surface with mercury.

The housing may further include a water-soluble membrane disposed between said tracer-containing means and said releasing agent-containing means. The water-soluble membrane is capable of being dissolved upon contact of said membrane by said sample and said tracer. The membrane may be of a thickness such that the membrane is completely dissolved after a predetermined time period. This predetermined time period provides for an incubation period of sample and tracer on the tracer-containing means.

In another embodiment, the tracer-containing means and the releasing reagent-containing means are separated by a space. As with the water-soluble membrane, the spacer provides for an incubation period of sample and tracer on the tracer-containing means. At the end of the incubation period, the device is compressed so as to enable the tracer-containing means and the releasing reagent-containing means to contact each other and to enable the flow of sample from the tracer-containing means to the dissociating reagent-containing means.

It is understood that the above-described water soluble membrane and spacers are also applicable to an embodiment utilizing a mercury releasing tracer in conjunction with a mercury containing substrate to

provide, for example, an extended incubation period for tracer and/or substrate reactions.

In alternative embodiments, the housing may further contain means for dividing sample flow into two paths. In one embodiment, a first flow path includes a tracer and binder, a releasing reagent, and a metal electrode as described above. The second flow path includes a tracer, a binder, a predetermined amount of mercury label independent of ligand, and a metal electrode. In a preferred embodiment, the first flow path also includes a spacer means, such as a blank pad, in order to balance the flow time of the sample paths.

In another embodiment, having a divider which divides the sample flow into two paths, the first flow path includes a tracer and binder means, a releasing reagent, and a metal electrode. The second flow path includes a predetermined amount of analyte, a tracer and binder means, a releasing reagent, and a metal electrode.

In yet a further embodiment, a first flow path in the device includes binder, tracer, releasing agent, and electrode. The second path includes tracer, releasing agent and electrode. The presence and/or amount of analyte is determined by comparing changes in the properties of the two electrodes; for example, such changes can be sensed electrically.

It is understood that the above-described self-referencing technologies are also applicable to the embodiment of the invention utilizing a mercury releasing tracer in conjunction with a mercury containing substrate.

As stated above, the device of the present invention may be used in connection with various types of assays, including competitive assays, sandwich assays, and indirect sandwich assays. Also, as above-mentioned, in most cases these assays require the use of at least one binder for the analyte in addition to the tracer. The exact method of placing binder and tracer on a releasing pad or pads within the sensor, and the order of contact of the tracer and binder by the sample depends, therefore, on the type of assay employed, and may vary within the scope of this invention.

Thus, for example, in a sandwich assay procedure, sample and tracer (a ligand which is bound by the analyte labeled with mercury) may be initially contacted in the capillary tube portion of the device to form a free tracer phase and a bound tracer phase in which tracer is bound to analyte. Thereafter, such phases are contacted with a binder for the analyte whereby the bound tracer phase is bound to the binder. The free tracer phase, which remains unbound then contacts releasing agent and released mercury interacts with the electrode.

The present invention is also applicable to DNA (RNA) determination in which case the tracer is appropriate DNA (RNA) labeled with mercury.

After hybridization, mercury is released from the hybridized probe and caused to interact with a metal, as hereinabove described.

Accordingly, the term "tracer" or "ligand labeled with mercury" as used herein includes a DNA (RNA) probe labeled with mercury.

The following are examples of the present invention. The scope of the invention, however, is not intended to be limited thereby.

EXAMPLE 1 - GLUCOSE TRACER

Polymer

A. Fluorescent Polyethyleneimine (PEI)

In a 200 mL beaker, add 20.00 grams PEI solution (50% aqueous solution, molecular weight of 50,000, Aldrich #18,197-B); add 80.00 mL distilled water, mix and/or sonicate until homogeneous. Add 10 mg (1 vial) lissamine rhodamine B (LRB, Molecular Probes #L1 908) to glass scintillation vial; add 0.5 mL dry Dimethyl Formamide; stir until LRB is dissolved. Add 4.5 mL distilled water rapidly; mix well; and transfer immediately to rapidly stirring PEI solution. Mix at room temperature for 15 minutes. Add 30.0 grams NaCl to solution and mix until dissolved. Filter through glass filter (Whatman 934AH). Dilute solution by adding 200 mL aqueous saturated NaCl solution; mix until homogeneous. Volume approximately 300 mL.

B. glycidol-PEG-Cl

In a 500 mL flask, add 100 mL dry acetone and 6.5 grams 1,1'-Carbonyldiimidazole (CDI, Aldrich #11,553-3); mix in water bath at 30°C for 15 minutes. Add 20.0 grams polyethylene glycol (PEG, molecular weight = 1,000, Sigma #P-3515) as a waxy solid. Stir for 15 minutes at 30°C. The PEG should dissolve within approximately 5 minutes.

- 5 To the above solution add 1.330 mL glycidol (1.482 grams, Sigma #G-0887) and mix for 1.0 hour at 30°C; evaporate acetone from solution at 30°C, under reduced pressure.

C. Graft polymer

- 10 Dilute glycidol-PEG-Cl solution from B above with 50 mL aqueous saturated NaCl. Mix rapidly. Add this solution to the PEI solution from A above while rapidly stirring. Stir for 2 minutes at room temperature, then add 10.00 mL distilled water while continually stirring. Solution clarifies in approximately 2 minutes. Continue stirring for 10 minutes total, then add 100 mL ammonium hydroxide (Fisher #A669C). Stir for approximately 1 minute; add 5.00 mL 6N NaOH to raise pH to 12.0. Stir at 32°C for 20 hours.
- 15 Dialyze in Spectra/Por 1 tubing (D1614-4, molecular weight = 6-8,000) against distilled water of pH 10.7 (NaOH) for 10 days at room temperature and 2 changes per day. After dialysis, evaporate excess water from polymer solution under reduced pressure (40°C) to 200 mL final volume (50 mg/mL initial PEI concentration). Store at 4°C until used.

20 D. glucose tracer

In a glass scintillation vial, dissolve 0.5 gms maltose (hydrate, Sigma M-5885) into 2.0 mL dry DMF. To this solution, add 1.333 mL of a solution of .500 gms CDI dissolved in 2.00 mL DMF. Add with rapid mixing. Mix for 60 seconds; then add 10 mL distilled water; mix rapidly; then add to a previously prepared solution of 10

- 25 mL of graft copolymer (C above) diluted with 20.0 mL distilled water. Mix at room temperature for 1 hour; then dialyze as before for 2 days. After dialysis, evaporate as before to 25.0 mL total volume.

Dissolve 400 mg. Mercuric acetate (Fisher M-143 75797) into above solution. Adjust pH to 7.0 to 7.1 with addition of aqueous NaOH. This solution may be frozen until used.

30

EXAMPLE 2 - GLUCOSE ASSAY

35 Aluminum Preparation

The aluminum used was 1145-0 (Davidson Metals, Inc., Specialty Metals Division) at .005 inch thickness. A sheet approximately 3.5 inches by 9 inches is immersed in 600 mL of 1.0 M NaOH (EM Science) in distilled H₂O and allowed to react for 5 minutes at 20°C. The foil is removed with forceps and thoroughly

- 40 rinsed with distilled H₂O. It is then wiped dry on both sides with a soft cotton cloth, paper towel or tissue. The foil is next immersed in 300 mL of a (92°C) solution of .42 M H₃PO₄ (JT Baker), .59 M CrO₃ - (Mallinckrodt for 10 minutes. It is then removed with forceps; the acid is allowed to initially drain off then it is thoroughly washed with distilled H₂O. The foil is then wiped dry as in the previous step.

- 45 The last treatment immerses the foil in 300 mL of a boiling solution of 10% (w/v) methoxy-polyethyleneglycol, molecular weight 1900 (Sigma) for 10 minutes. It is removed with forceps; washed thoroughly with distilled H₂O and wiped dry.

The prepared foil is stored under ambient conditions in plastic bags.

Sensor Base Assembly

- 50 The base for the sensor is assembled by utilizing a disposable glass microscope slide (Abco), 1" by 3". This provides rigidity and support for subsequent manipulations.

To this is applied 2 strips of double stick tape (Scotch) which cover the entire surface as well as overlaps one end by approximately 1/2". The treated foil is then applied to the tape and the slide is pressed onto the foil. The 1/2 inch overlap is then cut approximately .33 inches on both sides of the slide and folded over to form a contact point for attachment of a clip to the foil. The foil plate will become the negative electrode in the system. The foil layer is insulated from the positive electrode by overlaying No. 33 electrical tape (Scotch), .007 inch thick, through which 6, .245 inch diameter holes have been punched that are .5 inch

apart, center to center. The tape is pressed down to insure a seal around the edges of the holes. The positive electrode is attached by using the same treated aluminum which is cut into small pieces .05 inch wide by .75 inch in length. The positive electrode is then placed onto the insulating tape and overlapped onto the exposed aluminum by .025 inch. The positive electrode is held in place with a plastic self-adhesive, .26 inch diameter reinforcement (Avery Label). An overlap of .008 inches occurs around the insulating tape.

Releasing Pad Preparation

The component used to release the mercury ion from the glucose tracer is prepared by manufacturing .245 inch diameter circles of Eaton Dikeman 937-20 paper (.010" thick). A solution of .1M $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Sigma) plus, 5.0 M NH_4Cl (Fisher) is prepared in distilled H_2O . The paper circles are then placed on a 1" by 3" disposable glass microscope slide (Abco) in rows 3 by 10. To each of these circles, .007 mL of the $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}/\text{NH}_4\text{Cl}$ solution is dispensed. The entire glass slide is placed in a 60°C oven and allowed to dry for 30 minutes. The releasing pads are then stored desiccated at 20°F.

Concanavalin A/Glucose Tracer Pad Preparation

The component used to selectively react with glucose is prepared by manufacturing .245 inch diameter circles of Eaton-Dikeman 939-39 paper (.028" thick). The paper has a "coarse" and a "smooth" side. The circles are placed, coarse side down, on a disposable polystyrene petri dish (Fisher).

The solution applied to the paper is made by dissolving Type V Concanavalin A (Sigma) at .05 g/mL in distilled H_2O . The glucose tracer is diluted 20% with saturated NaCl (Sigma). The components are added to a 12 by 75 mm disposable glass test tube (Scientific Products) as follows: 1) 1.0 mL Concanavalin A (50 mg/mL), 2) .286 mL of distilled H_2O , and 3) .857 mL of the NaCl diluted glucose tracer. The components are mixed and then allowed to react for 15 minutes at 20°C. The solution is then dispensed onto the previously prepared paper circles at .0175 mL/circle. The plastic petri dish is then placed in a dry ice/isopropyl alcohol (JT Baker) bath and allowed to freeze thoroughly (30 Minutes). The paper disks are then lyophilized for 18 hours. Subsequent storage of the prepared paper is at 20°C, desiccated.

Final Sensor Assembly

The sensor base plate has added to each of the .245 inch diameter exposed aluminum surface, a single releasing pad paper circle. The releasing circle is overlaid directly on the aluminum surface (negative electrode) and the small positive electrode is repositioned on the upper surface of the paper. When dry, the releasing circle acts to insulate these electrodes from one another. Directly on top of the releasing pad and positive electrode, the Concanavalin A/Tracer pad is directly overlaid.

Performance of Glucose Assay

The assembled sensor is connected to the electronic circuitry by a negative electronic lead, to the common aluminum surface and, positive lead, to the small individual strip.

The electronic circuit maintains a constant .200 volts between the positive and negative electrodes, averages the current flow between electrodes for 60 seconds after blood addition and displays the average value in microamps.

Glucose in whole blood is measured by compressing the paper pads together with forceps, and, while maintaining this pressure, dispensing .04 mls of whole blood onto the Concanavalin A/Tracer pad. Pressure is maintained for 1 minute while the current generated is measured.

A typical standard curve for glucose is shown below in tabular form:

Glucose Concentration (mM)	Measured Current (uA)
0.0	2.4
6.0	8.6
12.0	17.4
24.0	34.6

Electrode Preparation

A procedure for treating each aluminum electrode includes treating the electrode in order to remove Al_2O_3 and other impurities, allowing restoration of the Al_2O_3 and $Al(OH)_3$ layers under controlled conditions, and then adding a wetting agent to the electrode surface so as to make the surface uniformly hydrophilic. The surface layers of oxide hydroxide of the electrode should be kept sufficiently thin so that it can react properly with the released mercury ions.

A preferred treatment of the aluminum electrode is as follows:

Aluminum is reacted in approximately 1.0 molar sodium hydroxide solution for 5 minutes at room temperature in order to dissolve the surface of the aluminum, as well as to remove any impurities. The aluminum is then rinsed with deionized H_2O .

The aluminum is then reacted in 0.4 molar Cr_2O_3 with 0.6 molar H_3PO_4 for 10 minutes at 88° to $92^\circ C$. This removes the oxide layer from the aluminum and neutralizes the surface, which had been basic as a result of the previous addition of sodium hydroxide. The aluminum is then rinsed again with deionized water, and then boiled in 0.05 molar methoxy polyethylene glycol solution for 10 minutes. Methoxy polyethylene glycol serves as a wetting agent for the surface and provides a uniform hydrophilic Al_2O_3 and $Al(OH)_3$ surface. Although methoxy polyethylene glycol is a preferred wetting agent, it is to be understood that other wetting agents may also be employed, e.g. PEG, surfactants, polyhydroxy compounds, etc. After the aluminum is boiled in the methoxy polyethylene glycol solution it is air dried. The product is an aluminum electrode or plate having a uniformly hydrophilic surface layer having a proper sensitivity for interaction with mercury. Although the above treatment procedure is illustrative of the preparation of an aluminum electrode having a surface layer of a desired thickness, it is to be understood that other procedures may be employed and that the scope of the invention is not to be limited by the above teaching. It is believed that the wetting agent treatment fulfills two purposes. First, it provides a strong wetting agent near the surface, and second, it acts as a porogenic agent, causing small pathways or cracks (voids) in the $Al(OH)_3$ layer.

Sensor Signals

As explained earlier, the tracer released mercury interacts and ultimately amalgamates with the surface of the large negative aluminum electrode, of the sensor as a function of the quantity, if any, of test analyte present in the liquid specimen introduced into the bioassay instrument. The interaction and ultimate amalgamation changes the surface characteristics of the aluminum electrode, which changes can be measured electrically, producing current signals, which are representative of the quantity of test analyte in the specimen.

Typical samples of amplifier current signal waveforms from a single output sensor for the analyte glucose are shown in Figures 18 and 19. The area under each curve is related to analyte concentration.

Empirical studies have shown there is a monotonically increasing relationship between the sensed current and the released mercury concentration in the processed specimen. The relationship is graphically shown in Figure 20.

Two sensor output waveforms result, when a dial output sensor is used, each of which is amplified. The relationship between the two output wave forms is a measure of the quantity of mercury released from the free tracer and, thus, a measure of the concentration of test analyte (glucose, for example) present in the liquid specimen.

The invention may be embodied in other specific forms without departure from the spirit or essential characteristics thereof. The present embodiments, are, therefore, to be considered in all respects as

illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalence of the claims are therefore to be embraced therein.

Claims

1. An assay for determining an analyte characterized by the steps:
forming in the assay a bound tracer phase and a free tracer phase, said tracer being a ligand labeled with mercury;
releasing mercury from at least one of the bound and free tracer phases;
interacting released mercury with a metal; and
determining analyte by determining a change in at least one property of the metal caused by said interaction.
2. The assay of claim 1 wherein said tracer further characterized by a carrier group comprised of a polymer comprised of a first portion, said first portion being attached to said mercury label and a hydrophilic second portion, said second portion being attached to said ligand.
3. The assay of claim 2 wherein said mercury label is further characterized by a plurality of mercury atoms attached to said carrier group.
4. The assay of claim 3 wherein said first portion of said copolymer is a polyethyleneimine, and said second portion of said copolymer is a polyoxyalkylene.
5. The assay of claim 4 wherein said polyoxyalkylene further includes an end group, said end group being capable of being linked to the ligand portion of said tracer.
6. The assay of claim 5 wherein said end group is an epoxy group.
7. The assay of claim wherein said assay is a competitive assay.
8. The assay of claim 1 wherein said assay is a sandwich assay.
9. The assay of claim 1 wherein said assay is an indirect sandwich assay.
10. The assay of claim 1 wherein the analyte is DNA (RNA) and the tracer is a DNA (RNA) probe labeled with mercury.
11. An assay kit for determining analyte characterized by:
a tracer for said analyte, said tracer comprising a ligand labeled with mercury; and a releasing agent for releasing mercury from the tracer.
12. The kit of claim 11 and further comprising a binder for at least the analyte.
13. A product for use in determining analyte characterized by:
a tracer for said analyte, said tracer comprising a ligand labeled with mercury;
a binder for at least said analyte;
a metal, said metal capable of interacting with mercury to change at least one property of the metal;
a releasing agent for releasing mercury from the tracer; and
a housing containing said tracer, said binder, said releasing agent, and said metal positioned in said housing such that upon adding of sample to said housing, there is formed a bound tracer phase and a free tracer phase, with said mercury label being released from one of said free or said bound tracer, causing said mercury to come in contact with and interact with said metal.
14. The product of claim 13 wherein said metal is aluminum.
15. The product of claim 14 wherein said metal is in the form of an electrode.
16. The product of claim 15, and further comprising:
an inlet means for receiving a sample; and
support means for said binder, tracer and releasing agent, said support means providing for capillary flow.
17. The product of claim 16, and further comprising means for determining a sufficient volume of sample within said inlet means.
18. The product of claim 16, wherein the support means comprises a first support means containing the tracer and a second support means containing the releasing agent.
19. The product of claim 16, and further comprising:
means for dividing the housing into a first flow path and a second flow path, said first flow path and said second flow path including binder, releasing agent and tracer and terminating at said electrode.
20. The product of claim 19, wherein:
one of said first and second flow paths contains a predetermined amount of mercury.
21. The product of claim 19, wherein:
at least one of said first and second flow paths includes a predetermined amount of analyte.

22. The product of claim 19, wherein:
one of said first and second flow paths includes no binder.

23. An assay for an analyte characterized by the steps of:

forming in the assay a bound tracer phase and a free tracer phase contacting at least one of the bound and
free tracer phase with a substrate including immobilized mercury, said tracer including a label which
interacts with the substrate to mobilize mercury;
interacting mobilized mercury with a metal; and
determining analyte by determining a change in at least one property of the metal caused by said
interaction.

24. The assay of claim 23 wherein the mercury in immobilized form is linked to a polymer and the
mercury linked to a polymer is contacted with a releasing agent to release mercury from the polymer prior
to interaction with the metal.

25. A product for use in determining analyte characterized by:

a substrate including mercury in an immobilized form;

a binder for at least the analyte;

a tracer including a label which interacts with the substrate to mobilize mercury;

a metal capable of interacting with mercury;

a housing containing the substrate, binder, tracer and metal positioned in said housing such that upon
adding sample there is formed a bound tracer phase and a free tracer phase, with free tracer phase
contacting substrate to mobilize mercury and mobilized mercury contacting and interacting with the metal.

26. The product of claim 25 wherein the substrate is characterized by mercury linked to a polymer and
said immobilized mercury is linked to said polymer, said housing further including a releasing agent for
releasing mercury from the polymer prior to contact of mercury with the metal.

27. A tracer of use in an assay as defined in claim 1 characterized by:

a copolymer of a polyimine and a hydrophilic polymer, said hydrophilic polymer being conjugated to a
ligand, said polyimine having attached thereto a plurality of mercury ions.

28. The tracer of claim 27 wherein the hydrophilic polymer is a polyoxyalkylene and the polyimine is
polyethylenimine.

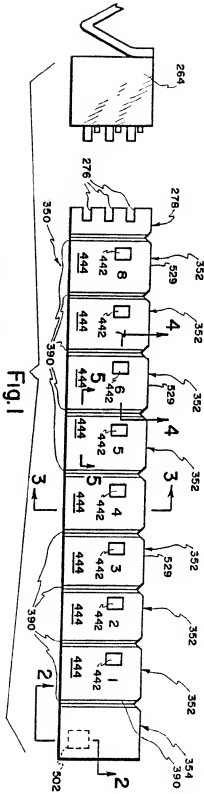


Fig. 1

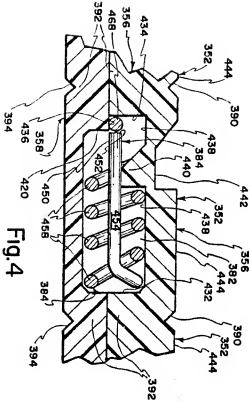


Fig. 4

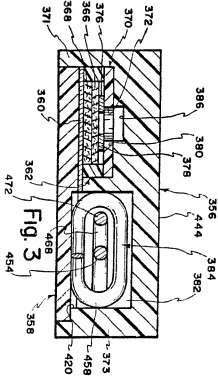


Fig. 3

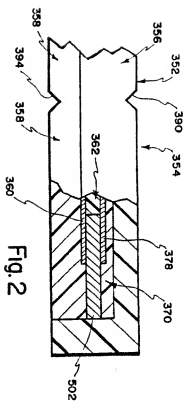


Fig. 2

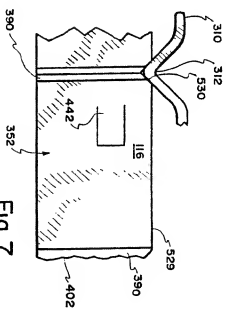


Fig. 7

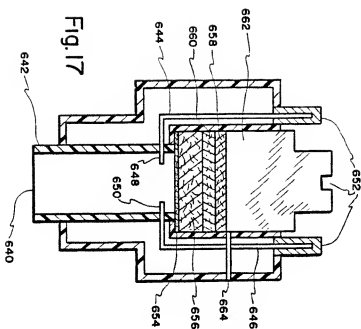


Fig. 17

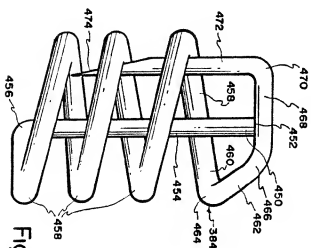


Fig. 9

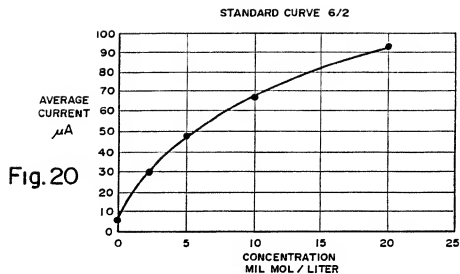


Fig. 20

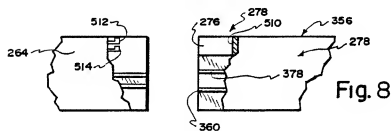


Fig. 8

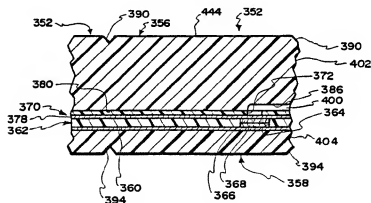


Fig. 5

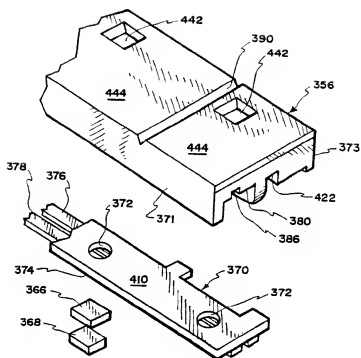


Fig. 6

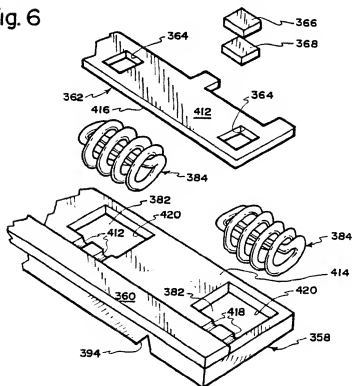


Fig.10

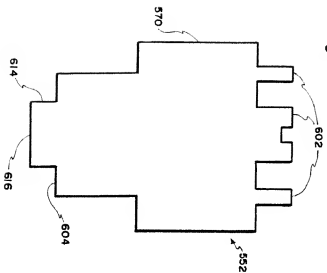


Fig.11

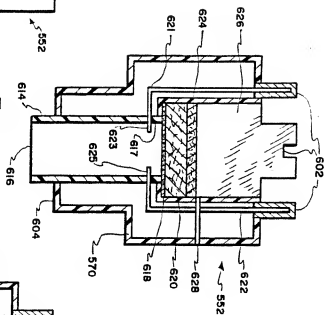


Fig.16

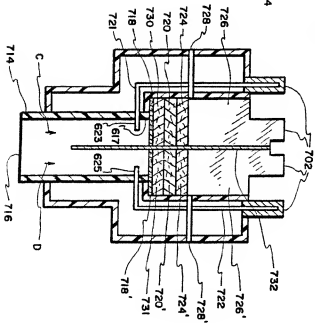


Fig.12

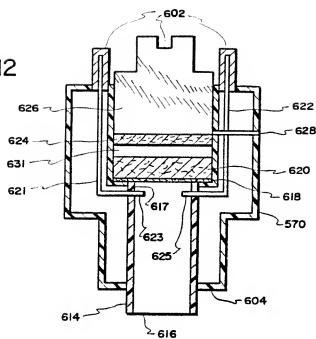
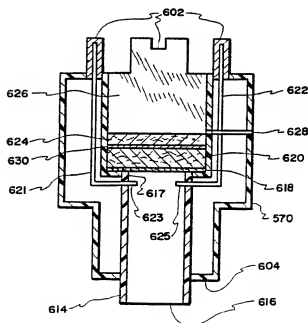
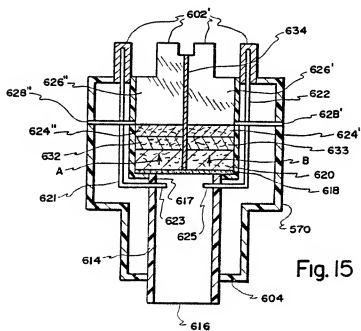
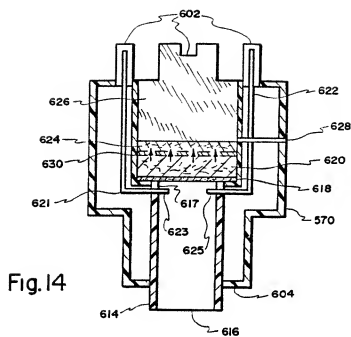


Fig. 13





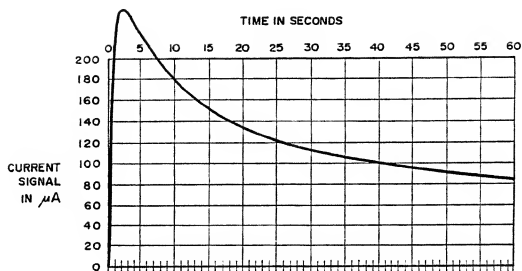


Fig. 18

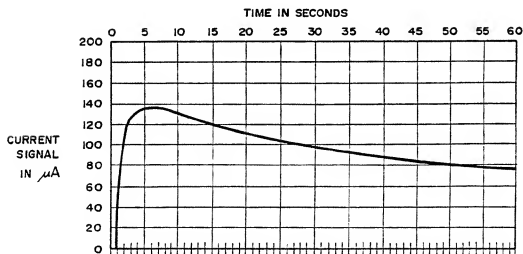


Fig. 19



DOCUMENTS CONSIDERED TO BE RELEVANT			EP 89310342.4
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
A	<u>US - A - 4 313 734</u> (LEUVERING) * Claim 45 *	1,7,8, 9,11, 23	G 01 N 33/58 G 01 N 33/553 G 01 N 33/66 C 12 Q 1/68
A	<u>US - A - 3 853 467</u> (GIAEVER) * Abstract; claims 1-7 *	1,13- 15	
A	CHEMICAL ABSTRACTS, vol. 107, no. 1, July 6, 1987, Columbus, Ohio, USA A.H.N. HOPMAN et al. "A non- -radioactive in situ hybridi- zation method based on mercurated nucleic acid probes and sulfhydryl-hapten ligands" page 380, Abstract-no. 3 488d & Nucleic Acids. Res. 1986, 14(16), 6471-88	1,10	
A	<u>DE - B2 - 2 657 351</u> (MILES LABORATORIES, INC.) * Claims *		TECHNICAL FIELDS SEARCHED (Int. Cl. 4) G 01 N 33/00 C 12 Q
A	<u>DE - A1 - 2 551 004</u> (OWENS-ILLINOIS) * Claims *		
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 18-12-1989	Examiner SCHNASS
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			